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Thank you very much. I would like to thank the organisers for inviting me to come to this meeting, it's a very interesting format.

I would like to talk to you today about the research that we have been conducting for about the last 10 years. The principal objective when we started, and continuing today, has been to make inexpensive, oral vaccines that will be suitable especially for the developing world, but also of course for the developed world.

I'll give the conclusion first. At the end of a decade's worth of work we have completed three human clinical trials. In every case, we have started our human clinical trials by bringing genetically modified food, in this case transgenic potatoes, to a clinical site where the human trials can be conducted. All of our trials have been conducted with the full approval of the U.S. Food and Drug Administration (FDA), following the standard regulatory approvals for any new experimental drug. Of the trials that we have conducted, two of them have been at the National Vaccine Testing Centre, which in the U.S. is one of our speciality research centres for oral vaccines. We also have conducted one trial, in this case for hepatitis B vaccines, at the Rosswell Park Centre in Buffalo, New York.

We've done successful clinical trials for two prototype vaccines to prevent diarrhoea. In one case bacterial and in the other case viral. In every case we start the experiment in Baltimore. In this case it's the head nurse that initiated the experiment. She brought her potato peeler to work, peeled the potatoes and diced them up. We washed them, put them in a little plastic bag and that's the means of dispensing the vaccine. The human volunteer in this case simply sat and ate about 100 grams of raw potatoes.

We have now had a total of 82 human volunteers in the various experiments. The number is largely chosen due to cost issues. If nothing else these trials are fairly expensive to conduct. The volunteers eat either 50 grams or 100 grams of our transgenic potatoes. We've never seen an adverse effect and in every case we've seen humeral and mucosal immune responses.

Stepping back to where we were about a decade ago, how do we create these plants in the first place? Well, we are using standard technology, such things that are done in good high school biology labs these days, by cloning a gene and in our particular case the gene of choice was the hepatitis B surface antigen. Now why did we choose that gene? It is the gene that is used for the commercial vaccine, which is sold in most parts of the world. However, it is not widely used in all parts of the world. It is not used here in the UK because the cost benefit analysis has not caused the British Health Authorities to demand hepatitis B immunisation. In the U.S. it is a required paediatric vaccine for immunisation; so for kids at schools and colleges have to have their hepatitis B immunisation. At the present time the hepatitis B surface antigen product is the gold standard for the new generation of recombinant vaccines which really started in the mid-1980s. It is incredibly safe, its very effective, and the commercial vaccine today is produced in yeast. It is grown in fermentation, and then there is about a 12-step purification process which results in the U.S. of a single dose of the hepatitis B vaccine being about 75 dollars a dose. Since our government agencies or our

insurance systems pay for it, 75 dollars isn't bad.

If you think about getting these modern vaccines to the developing world, however, you have got to look at what vaccines are widely used around the globe and a good measuring point is the measles vaccine, which is not globally available because of cost. Measles vaccines cost 10 cents a dose and that is sort of a good bench mark established by the World Health Organisation as a cost level that you have to get below if you are going to have penetration on a global basis.

We have very good ways of making hepatitis B vaccine today that gives us a product that is affordable in the U.S., Europe, Japan, but it does not get to 40% of the world's population even with a large amount of philanthropy by the Gates Foundation and many other organisations. 40% of the world's population doesn't get this effective vaccine because of cost. So because of those two
reasons we decided to put the gene instead of into yeast transferred into a plant cell and then regenerate the plant.

Now one of the beauties of transgenic plants is that they do arise from a single cell receiving a gene, and then that gene becomes implanted in the chromosome and so every cell of the resulting plant contains that new gene and every cell therefore has the capacity to manufacture that new protein. What we have done (this happens to be potatoes now) was transfer genes into potatoes in our
first experiments, and then grew the potatoes, harvested some potatoes, fed them to mice, went through all the preclinical trials and showed that, in fact, they were orally active when they consumed this food. That was without any processing step whatsoever.

We presented that data to the FDA in the U.S. It took us about six months to get the first approval, that's a fairly long time for a vaccine. But the FDA had never seen a product like this before, none of our production technology fit with standard protocols. It really took a clever person within the FDA to say we've been using the wrong forms, we've been looking at all the forms for drug approval, this is a food additive, lets look at food additive regulations. Once that was recognised a couple of months later we did get approval to go through this process and, of course, we've not seen any adverse effects, we've only seen positive effects from it.

We had human volunteers eat our potatoes. If they eat all normal potatoes of course nothing happens. Two doses gave a response, but three doses of potatoes gave a very, very substantial response. I won't bore you with the details as it happened to be a boosting trial for a variety of technical reasons we did it that way. But the simple point was that we can now get a very good immune response in humans with a transgenic potato which contains a single protein of the hepatitis B virus, the same protein which you receive normally by an injection in your arm. We have done the same experiments now for two other diarrhoeal vaccines with the same sort of data, so we have been able to successfully immunise humans. That's all the data that I'm going to give you on the scientific side of the immunisation and creation of these plants.

I started this with a naive notion that once we had got this technology in place we could take a plant material, not raw potatoes but a plant material, and distribute it on a global basis as some sort of vaccine. I naively early on had an image of a village banana tree where people would go to get immunised. As I

began, and I'm a plant biologist who came out of a agricultural background, speaking with the folks who are involved in the pharmaceutical industry, it became abundantly obvious that we weren't going to get a licence for our products if we go that way, and we have to deal with dosage issues, timing issues, and we had to treat this as a medical product. So we've switched gears in the last two or three years and began to focus our energy on aspects of production of the vaccine and processing to make a stable product, antigen stability, shelf life for the material. If you're going to load it onto a boat and take it up the Congo River, for instance, is it going to be stable, is it going to arrive in good shape?

This aspect of shelf life is a major limiting factor for current vaccines, which need to be refrigerated from point of manufacture to point of use. What we are trying to do is get rid of what's called the cold chain, or the requirement for a refrigeration step, because often times just the refrigeration component costs more than the actual vaccine does when you are taking these products
into remote parts of the world

Now as we get into these issues of production and processing I've begun also to focus my energy on how we are going to get these things licensed in the U.S. This is not because I think that the U.S. is going to be the primary place for it to be done, but as I'll come back to you in the end, we want to make sure that we can get regulatory approval in the U.S. so that we aren't accused of taking these materials some place else and experimenting on poor people in poor countries.

We have participated now with USDA and FDA, which is actively involved in the development of new regulations on plant based manufacture of macro-molecular drugs. Several people from our group participated at the first conference in Iowa, which was jointly sponsored by USDA and FDA. After that, various actions and work committees were established which are still operating. The early products, which the action committees are considering, are plant-based manufacturing, first for monoclonal anti-bodies, for which there are now or at least potentially at least six products in human clinical trials, most of them are monoclonals, which are intended to be used for cancer therapy. I can come back to why plants if someone is interested in that later with respect to monoclonal anti-bodies.

The next products which will probably hit the regulatory entry point will be vaccines for animal agriculture and I would anticipate we are doing some of this research. The first products here will be going in for license in the next two to three years. Therefore, there is a pressure point in the U.S. to get this regulation in place to deal with these new commercial products that will be entering the product stream. FDA regulates human vaccines and USDA regulates animal vaccines in the U.S. FDA is preparing for a license or a procedure that we would like to take through sometime in the next two to three years.

Now there are various issues that remain that have to be established, and one of the principal ones is when does manufacturing start? When do we deal with good manufacturing practice? Which is the regulatory framework to ensure the way and the quality that a product is regulated through the manufacturing?

Let me step back now, our real goal in all this is to develop inexpensive vaccines where the manufacturing capability could be done in the developing world. We don't want to develop a technology that requires complex chromotography, protein purification and all the hallmarks of highly sophisticated vaccine manufacture as its conducted in the U.S., in Europe and various other parts of the world.

So if we are starting with plant material our goal has been to take technology that already exists, and in particular food processing techniques such as freeze drying, dehydrating, juicing, spray drying, and pulping. Banana puree is a pulping process this is freeze dried tomato juice, dry banana slices instant mash potatoes. These are technologies that are in place today and are used around the world. Let me give you the specific example of potatoes. We are not going to try to get little kids in the developing world to eat raw potatoes, that's pointless, but the technology easily exists today to make something equivalent to instant mash potatoes.

I'll use U.S. examples not because this is the way I think this should be done but rather the examples are there. Any potatoes that go into this sort of process products are contract produced. A company works with a farmer and contracts for a specific type of potato to be grown. They can harvest and store those products for up to 12 months. Once they get ready for processing they wash them. The way that potatoes are peeled in most parts of the world for industrial processing is to simply drop them into caustic lye. It just burns off the peel and you end up with a peeled potato very nicely without damaging any interior part of the potato and then its washed and neutralised and then sliced and ground and in some cases freeze-dried to make this sort of a product.

What we have proposed in working with the regulatory agencies is that good manufacturing would start at this point. It's a logical point to start where you could use agricultural technology and down stream from that you would follow standard protocols that would be used in the pharmaceutical industry, except that now with a different type of product.

To test this we have also generated transgenic tomatoes with all of our different vaccines. This experiment was conducted in collaboration with individuals at the Cornell Food Science Department. In our greenhouses at the Boyce Thompson Institute we had 30 tomato plants last summer. Once a week we would go through and harvest ripe tomatoes. We would wash them, walk down the street and load them into this prototype industrial mixer/slicer which is in the food science department, grind them up, take the tomato puree that came out of it, pour it into stainless steel trays to be pre-cooled, and slide them into an industrial freeze dryer. It took about three days to dry them down.

From 30 plants, and a once a week collection of material, we generated 4,000 doses of a hepatitis B vaccine with 2 milligrams of hep B surface antigen per dose. We know that is accurate from our human clinical trials at that level of vaccine. I don't know exactly what that particular experiment cost us but we can make some calculations of cost based upon processing tomato technology. Now these are again U.S. costs, just because I wanted to take a bench mark. I can guarantee you this would be much lower if we were doing it in Bangladesh or Cambodia or Brazil or other parts of the world. In the U.S. we grow tomatoes primarily for fresh market tomatoes or processing tomatoes and since we are really dealing with processing issues here let me focus on this. At the farmers gate, the farmer gets about $54 per tonne, that is about 5 cents per kg of the actual tomato. Based upon the levels of expression that we've got and thus far without finally optimising the material, that means that we can produce 4 doses or 8 mg of hep B vaccine for one cent. I think that we are already down a factor of 10 over other manufacturing techniques. Now I will emphasise that these calculations that I've put here exclude any cost of processing, quality assurance package etc.

I want to make my disclaimers. What I'm trying to say is that the initial starting point in this can be very inexpensive and its our obligation now to develop the next steps in these, including quality assurance testing to make sure that we keep the costs low. Some of you may have heard me speak before. I have for the last eight years been dedicated to trying to do this with bananas. Bananas are an extremely difficult crop. When we started with bananas in 1992, no one had ever put a gene into bananas no one ever cloned a gene from bananas. There were no promoters for the fruits, there was nothing.

We have now built a molecular tool box that contains all the elements. We have proved that last summer by harvesting blue bananas, which is irrelevant from the vaccine point of view. What we did, however, was put in a gene that causes a blue dye to accumulate in this tissue, and so we can show that it in fact works. We had quite a celebration. We recently published this, and we were running around saying "yes we have blue bananas" and all that.

We have the technology, why would we want to do that? Well, bananas are grown in most developing countries of the tropics and semi-tropics. Bananas are a crop that is male-sterile, so it doesn't produce pollen, it doesn't out-cross, it is a crop that is eaten uncooked, uncooked is very important as all the vaccines that we are dealing with would be destroyed in the cooking process. Lastly it is a food that is very mild or bland in its taste and is very often given to young children.

We can imagine now as we are moving into the next step in our banana project we could develop process technology for making a banana baby food puree, based upon extracts of our levels of expression thus far. We could probably get 10 doses of vaccine in our baby food jar of that size, so it's about a tablespoon per dose if things continue to work as well as they have with potatoes and tomatoes. Or, of course, we can simply peel the material and dry it and make banana chips, which could be ground up into a powder.

Let me end up with our strategy of where we think we are going with this technology. We at the present time are focusing on getting at least one plant-based oral vaccine licensed in the U.S. and I hope to do that with an oral hep B vaccine. For a variety of reasons. We've got a very good vaccine on the market which is injectable and we can use various rules of equivalence where there is a good bench mark for what sort of immune response you need to get a protective immunity. So we've got a lot of good reasons for doing that. We want to do that in the U.S. because I just want to make sure that we can prove that this works in the U.S. Secondly, we are co-operating with scientists in other countries to develop parallel technology. My colleagues in Irapuato, Mexico, are Miguel G omez Limb, in particular, and his colleagues. We have worked together jointly for the last six years now on developing parallel technology and the labs now have plants with a variety of edible vaccines.

We believe that this technology will be developed in a number of other countries. There is also active research going on in China and India in the same areas, and we have some interaction with their researchers. We also want to conduct human clinical trials in international locations. We have established trials in Cuarnavaca Mexico where there is a very good vaccine immunology group and so we can validate and extend the studies in a different location. And lastly we are co-operating and collaborating with international groups especially the World Health Organisation. A group of us organised a meeting about a year and half

ago in Geneva to review the current state of this technology and work with them to bring the folks up to speed in Geneva, and to let them know what sort of technology advancements are coming. Three weeks ago in Washington there was another meeting that the World Health Organisation also sponsored and part of the meeting dealt with these issues.

Let me end up with a vision, its really pretty simple. We want to create a technology that will allow vaccine manufacture in the developing world. I probably should have started with this, but I think that most of you know, and let me remind you anyway, almost all of the vaccines that are used today throughout the world are manufactured in the U.S. or Europe or in the developed world. They are sold or given or subsidised as they get to the developing world.

I believe in philanthropy. I think it's fantastic but it's inherently unsustainable because philanthropic interests change over time. The Bill and Melinda Gates Foundation is probably the largest group right now buying vaccines for delivery. They are going to change their interest over time and probably want to do something else. Our real strategy should be to develop research that will be transferable so that developing countries can begin producing their own vaccines. It then becomes an economic incentive, creates jobs, employment, and a wealth generator, and becomes part of the economic stability of the country to maintain a public health system. It also reduces their dependency on international philanthropy and increases their own pride in public health systems.
So I think a sustainable vaccine programme requires transferring manufacturing technology into the developing world.

In addition to this, what we are doing is creating more oral vaccines for the prevention of a variety of diseases, which are under met vaccines and are not developed right now, in particular diarrhoea. This is not a big problem in the U.S. and Europe so we don't spend much money investing in these vaccines. If they are going to be practical, vaccines will need to be used frequently, probably boosted once every two to three years, maybe even once a year, and we've got to find ways to cut the cost to do that. And lastly I believe that this technology will be used to make inexpensive and effective animal vaccines, which is going to improve food safety and reduce the requirement for things like antibiotic use in the food supply.

With that I'll stop, thank you very much.